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THE EFFECT OF *Citrus sinensis* PEEL EXTRACT AGAINST *Rhipicephalus sanguineus* (Dog Ticks)

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ABSTRACT

Tick transmitted disease remains a major source of illness and death worldwide. Many chemical agents have been employed to control ticks. They are normally safe, but some of them may be responsible for toxic reaction in animals, reduced effect in the long run due to development of resistant strains of ticks, environmental pollution and human health hazards. Agricultural waste such as *Citrus sinenesis* peel has been exploited and highly potential as anti-tick. The purpose of this study is to assess the effect of *Citrus sinensis* peel extract against *Rhipicephalus sanguineus* commonly known as dog ticks**.** Sample sizes of 400 larval were obtained by tick breeding. Extraction of *Citrus sinensis* peels extract was obtained through two methods; heat press extraction and cold press extraction. For each method, the larvae were tested on 50%, 75% and 95% concentrations. Effect was measured in terms of tick mortality rate and rapidity of action. Maximum effect was found at 95% strength from samples obtained using the heat press method which shows 98% tick mortality rate with $P < 0.05$. The final results validated the insecticidal properties inherent in heat press *Citrus sinensis* peel extract.

Key words: *Citrus sinensis* and mortality rate of tick larvae in heat and cold press extraction

INTRODUCTION

Tick transmitted disease remains a major source of illness and death worldwide. Diseases associated with transmission of viruses to human from ticks are an expanding problem in tropical and subtropical regions (Ginsberg & Faulde, 2008). There are nearly 900 species of ticks' present in the world (Guilielmone *et al.,* 2003) and can cause a variety of health conditions ranging from harmless to serious.

Ehrlichia canis, a ubiquitous Rickettsiella pathogen of dogs, is the causative agents of canine *Monocytic ehrlichiosis*, the most common and one of the most clinically significant tick borne diseases of dogs in Malaysia (Nazari *et al*., 2013). A compilation study done by Mohammad *et al* (2016) also indicated these ticks can be found in domestic dogs as well thus making it possible for human contact. Humans can become infected by *E. canis* and other species after tick exposure. Clinical signs of human *Ehrlichiosis* include fever, headache, eye pain, and gastrointestinal upset (Paddock & Childs, 2003). While most ticks do not carry diseases, some ticks can carry bacteria that can cause disease to human such as Colorado tick fever, Lyme disease, Rocky Mountain spotted fever and tularemia. These tick bites cause flu, body- wide itching, chills, fever, headaches, muscle pain, stiffness and human will also develop severe red rash at the site of the bite where the tick disturb human healthy life style (Lane & Crosskey, 1995).

Ticks and tick-borne diseases effect animal and human health worldwide and are the cause of signicant economic losses both through the direct effects of blood sucking and indirectly as vectors of pathogens and toxins (Granstrom, 2000). Since, the danger of tick to the world is expanding, the use of chemicals as repellant or as an anti- tick is really necessary. Many chemical agents have been employed to control ticks. They are normally safe, but some of them may be responsible for toxic reaction in animals, reduced efficacy in the long run due to development of resistant strains of ticks, environmental pollution and human health hazards. As a result of these serious drawbacks, there is need to develop alternative, cheap, safe methods of tick control and mainly to prevent allergy towards

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human skin using dog as model. To avoid such adverse effects, plant or fruit derived products as repellants against ticks are preferred as alternatives (Ginsberg & Faulde, 2008), hence, the aim for the research is to study the effect of *Citrus sinensis* peels extract which is well known as orange peels extract as an anti-tick for dogs.

MATERIALS & METHODS

Selection of Sample

The selection of the sample are the *Rhipicephalus sanguineus* ticks. Total of 14 fully engorged ticks are collected from dogs around Selayang, Malaysia. The ticks were sent to Institute of Medical Research for the identification of the species and confirmed as *Rhipicephalus sanguineus*.

Breeding of Ticks

A total of 500 larvae of ticks of either sex was used. These ticks were produced using breeding method (Salleh *et al.*, 1982). Fully engorged females were separately placed in breeding pots which had a height of 3.8cm and diameter of 4.4cm. These pots were sterilized in boiling water before use. The pots, covered with cover gauze, were placed on trays resting over a tray of water and alcohol as a prevention method. Daily 3 drops of water were added using syringe to keep the pots moist until eggs hatched, ±45 days. Laboratory temperature during this breeding was room temperature. Daily samples were observed under dissecting microscope as to confirm the development stage of the ticks.

Plant Sample Desiccation and Extraction

A box of orange fruits was bought from a local market in Shah Alam and was sent to Institut BioSains, Universiti Putra Malaysia, Serdang, for identification and confirmed as *Citrus sinensis*. The oranges were rinsed with clean water and the orange peels were peeled and chopped with knife into small pieces. For heat pressing method, half of the peels were desiccated using a convection oven with a temperature of 50ºC for duration of 2 days. The dried peels were then crushed with the mortar and pestle. The crushed peels are then powdered with a blender to obtain a very fine powder. This powder was sieved again and larger bits were blended again until all peels were in powder form. The 250g powder was steeped in two liters of distilled water for 72 hours. Then the concoction was filtered using whatman filter paper 12.5cm. This was done before extracting to remove any peel residue. Then the filtered concoction was purified using rotary evaporator under pressure in a temperature of 60ºC. The extract yielded was in a thick orange color. For cold press extraction, roughly one kilogram of fresh orange peels was roughly crushed using mortar and pestle and extracted with use of pressure at room temperature. The extract yielded was in thick orange color. The cold pressed and heat pressed extract was then diluted in distilled water to achieve 50%, 75% and 95% concentration and were stored at room temperature.

Testing/ Experimental Study Design

A total of 500 tick larvae was split into 3 groups; control, cold pressed peel extract and heat pressed peel extract. Cold pressed and heat pressed extraction group was then each subdivided into three different concentrations which are 50%, 75% and 95%. For each concentration 50 larvae ticks were tested for each extraction method. Control group was divided into two; positive control and negative control. For positive control, marketing tick control spray was used and for negative control distilled water was used. For each control, 100 larvae ticks are used. For the three main groups, 5 larvae ticks are introduced into each Petri dish with different solution to test on the mortality rate and time taken for the larvae to die.

RESULTS & DISCUSSION

The results of this study showed that the orange peel extract obtained from the genus *Citrus sinensis* is a viable means of killing ticks in household pets. Based on the data presented in Table 1, it can be seen that the final kill rate is closely approximate to the positive control (~95%). However, effect of the final end product is not only dependent on the kill rate but also the rapidity at which the compound acts. In Fig. 1 and Table 1, the results clearly show that extracts obtained from the heat pressed method are far more efficacious. A mortality rate of 78% in the first 15 minutes of application versus the extracts obtained from the cold pressed method which yielded a paltry 18% within the same time frame. Heat pressed extraction shows fastest and high rate of effect which is similar to positive control.

The data compiled from this study yielded a comparison p-value of 0.399 for orange peel extract obtained through the heat pressed method within the first 15 to 30 minutes of application; whereas the cold pressed method yielded a p-value less than the valued obtained in heat pressed within a time frame of 45 to 60 minutes of application as observed in Table 2. Therefore, taken into account the final mortality rate and rapidity of action, extracts obtained via the heat pressed method yield maximum results within the first 15 minutes but the final kill count and overall potency is seen at the end of one hour obtained from the extract using the cold press method.

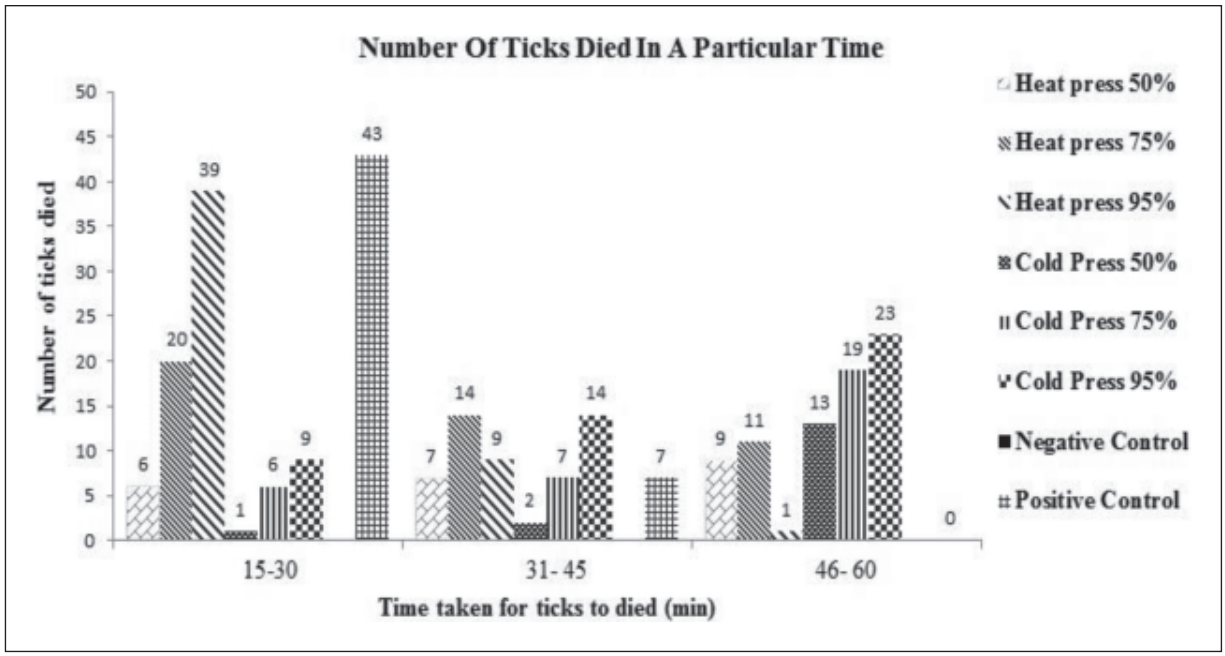

Fig. 1. The bar graph shows number of larvae ticks die in different concentration of different solution in 15–30 min, 31–45 min and 46–60 min.

Table 1. The mortality of ticks' larvae in each solution which includes heat pressed peel extraction, cold press peel extraction, positive control and negative control

Type of Extract	Concentration (%)	Total Numbers of Ticks Introduced	Time Taken for Ticks to Die			Mortality Rate (%)
			15-30 Min	31-45 Min	46-60 Min	
Heat press	50	50	6	7	9	44
	75	50	20	14	11	90
	95	50	39	9	1	98
	Positive control	50	43	7	0	100
	Negative control	50	0	0	0	0
Cold press	50	50	1	2	13	32
	75	50	6	7	19	64
	95	50	9	14	23	92
	Positive control	50	43	7	0	100
	Negative control	50	0	0	0	0

Min: Minimum

Table 2. The multiple comparison of anova between 95% concentration with other concentration in each method which give significant value

	Concentration 1	Concentration 2	SIG	Description
15-30 MIN HEAT	95%	50%	.000	No large different in numbers of ticks died between 95% and positive control.
		75%	.000	
		Positive control	.399	
		Negative control	.000	
46-60 MIN COLD	95%	50%	.186	Large different in numbers of ticks died between 95% and positive control.
		75%	.898	
		Positive control	.000	
		Negative control	.000	

Citrus sinensis peel contains higher concentration of active compounds such as d-limonene (Bourgou *et al*., 2012). Based on the data, it can be inferred that extracts obtained via the heat pressed method achieves better results overall in terms of kill rate and rapidity of action. The extraction method removes large parts of moisture and oxidizes unwanted oil resulting in a concentrated and potent yield versus the cold pressed method that yields a diluted extract that is inclusive of the large water content as well as non-essentials oils nascent within the orange peel. A feature achievable through low heat and extended time. The process also helps to achieve a greater oil yield by evaporating non-essential oil. The essential oil that was expected to be in present in the extraction was assumed to be there due to the results obtained in Table 2. The method for extraction using rotary evaporator had preserved majority of the essential oil contents rather than using centrifugation alone (Panneerselvam & Murugan, 2013). Using the rotary evaporator at much lower temperature yielded much more concentrated extract at almost 13.5 g worth from 500 g of plant material. Different methods have an effect on some of the components of citrus oil in accordance with previous findings (Ebrahimi *et al*., 2012).

The components obtained by heat press method were high because of the application of heating for appropriate duration resulting in high concentration of essential oil compounds. The higher proportion of the essential oil in heat pressed method was probably due to hydrolysis of some components that can react with water at high temperature and provide essential oil. Aldehydes are constituents of essential oils and, in the presence of water, especially at high temperatures; they tend to react with water to form concentrated oils. Oil components are sensitive to hydrolysis while others like acyclic monoterpene hydrocarbons and ester are susceptible to polymerization since the pH of water is often reduced during distillation when hydrolytic reactions are facilitated (Murugan *et al*., 2012).

In general, oven-dried citrus peels had higher oil yield followed by the fresh samples. The results of our present analysis regarding the effects of drying conditions on peel essential oil yield are in agreement with the findings of Kamal *et al* (2012). According to him, effects of drying on the yield and chemical composition of essential oil from the aerial parts of *Leonotis leonurus* was studied and found that the oils derived from oven-dried plant material had better yield than those from the fresh materials which were similar to this research's study. Some other reports in the literature also revealed considerable effects of drying on the yield and characteristics of the essential oils (Murugan *et al*., 2012).

CONCLUSION

In conclusion, *Citrus sinensis* peel can be used as insecticide against tick. *Citrus sinensis* peel extract is viable and safe tick repellant. *Citrus sinensis* heat pressed extract of 95% concentration shows the highest effect against ticks. In lieu of the natural origins and methods used to extract the active compounds themselves involves mainly purified water the safety profile of the extract itself is unmatched. Furthermore, the effect of the product is a close approximation to the positive control using the heat extraction method. Future efforts will be directed towards practical means of extracting and commercializing the product itself.

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